

Nucleic Acid-Based Tools for Monitoring Bioremediation at Chlorinated Solvent Sites

**Erik A. Petrovskis, Ph.D., P.E.
Geosyntec Consultants**

**Environment, Energy and Sustainability
Denver, May 2009**

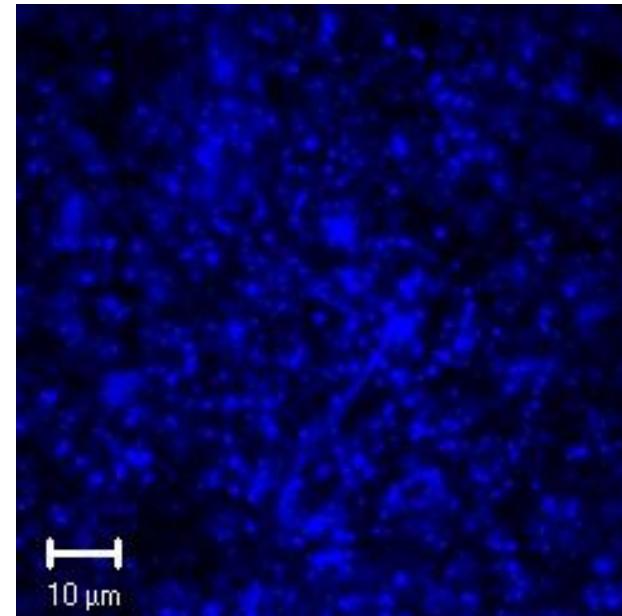
Report Documentation Page			<i>Form Approved OMB No. 0704-0188</i>	
<p>Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</p>				
1. REPORT DATE MAY 2009	2. REPORT TYPE	3. DATES COVERED 00-00-2009 to 00-00-2009		
4. TITLE AND SUBTITLE Nucleic Acid-Based Tools for Monitoring Bioremediation at Chlorinated Solvent Sites			5a. CONTRACT NUMBER	
			5b. GRANT NUMBER	
			5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)			5d. PROJECT NUMBER	
			5e. TASK NUMBER	
			5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Geosyntec Consultants, Ann Arbor, MI, 48105			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSOR/MONITOR'S ACRONYM(S)	
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited				
13. SUPPLEMENTARY NOTES Presented at the NDIA Environment, Energy Security & Sustainability (E2S2) Symposium & Exhibition held 4-7 May 2009 in Denver, CO. U.S. Government or Federal Rights License				
14. ABSTRACT				
15. SUBJECT TERMS				
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 31
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified		

- Learn the science behind MBTs
- Learn when to use MBTs
- Learn how to sample groundwater for MBTs
- Learn the “Rules of Thumb” for MBT data
- Learn how to save time and money with a smarter bioremediation design and operation– do I bioaugment?



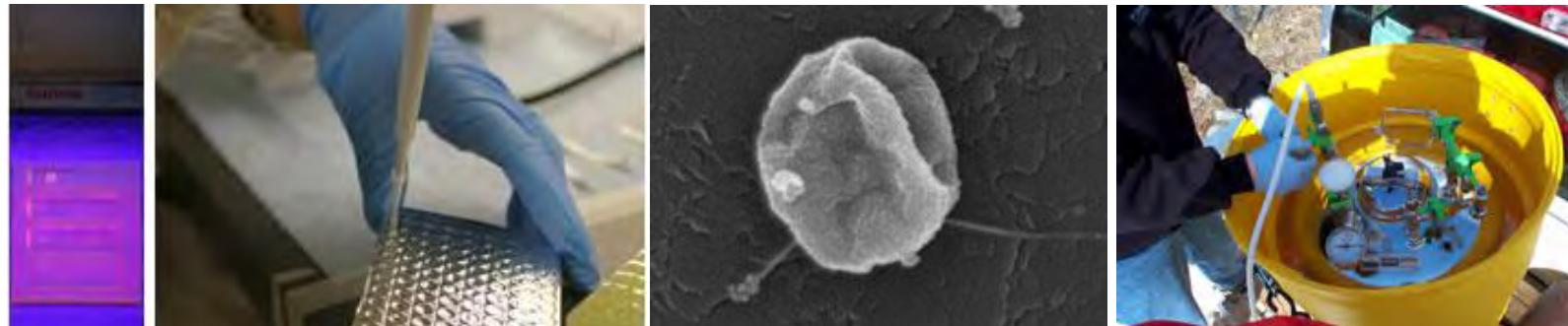
Types of MBTs

- **Nucleic acid probes (DNA or RNA)**
 - Various targets including
 - 16S rRNA gene
 - Functional genes (e.g., RDase, Hydrogenase, Oxygenase, etc.)
- **Protein biomarkers**
- **Lipid biomarkers**

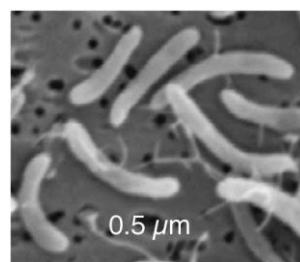
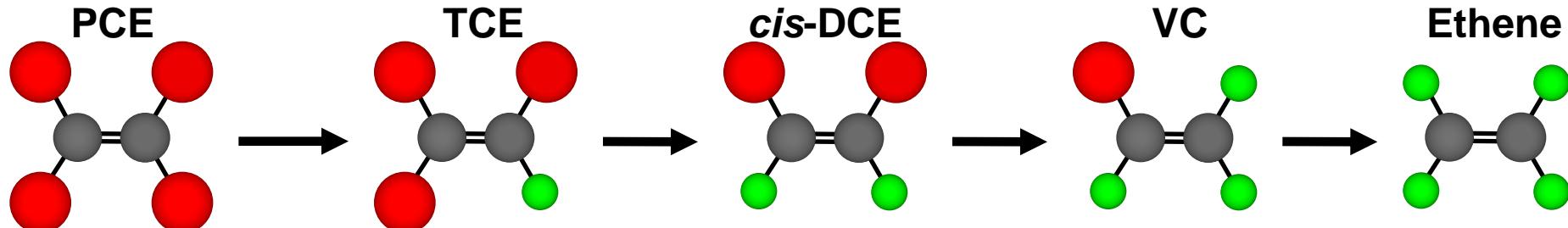


Purpose of MBTs

- Reduce remediation costs and increase effectiveness by
 - Supporting sites where MNA is being evaluated
 - Predicting sites where biostimulation will succeed
 - Identifying sites where bioaugmentation is required

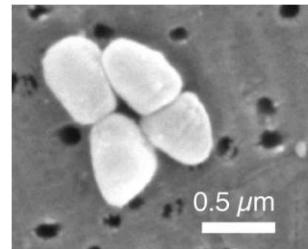


Chlorinated Ethene Reductive Dechlorination



Desulfitobacterium
sp. strain Viet1

Desulfitobacterium sp.
strain PCE1

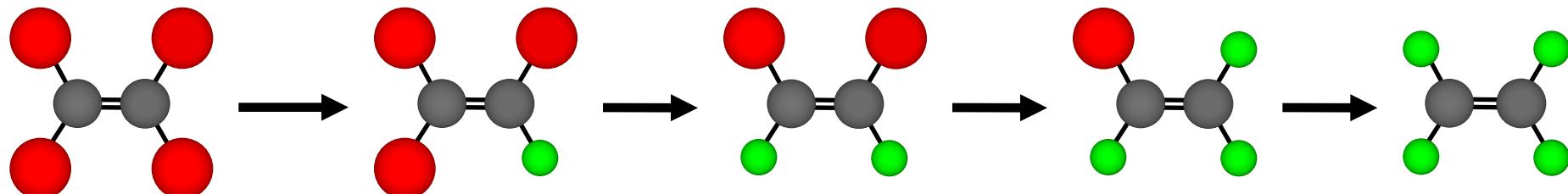


Desulfuromonas michiganensis

Sulfurospirillum,
Desulfitobacterium,
Dehalobacter, Geobacter



Dehalococcoides (Dhc) Involved in Reductive Dechlorination



Dehalococcoides ethenogenes strain 195

Maymó-Gatell et al. 1997. Science 276:1568

Dehalococcoides sp. strain FL2

He et al. 2005. Environ. Microbiol. 7:1442

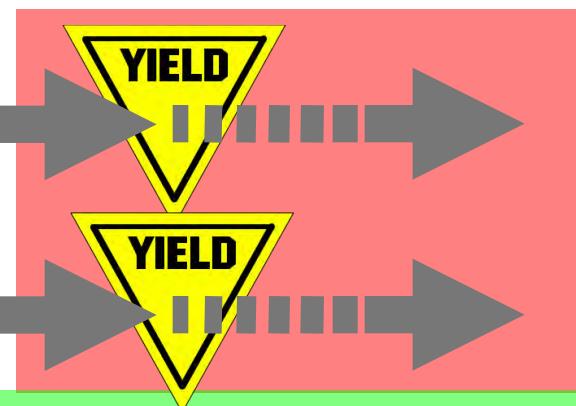
Dehalococcoides sp. strain BAV1

Müller et al., 2004, AEM, 70:4880

Dehalococcoides sp. strain VS

Sung et al., 2006, AEM, 72:1980

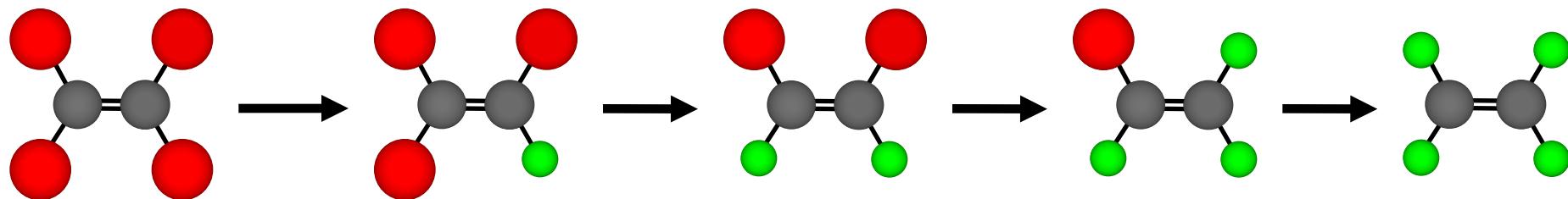
Dehalococcoides sp. strain GT



- 16S rRNA found in all bacteria
- rRNA part of the ribosome; critical for protein biosynthesis
- Contains variable regions which allows for the differentiation of bacterial species
- *Dhc* has one 16S rRNA gene per cell
- *Dhc* 16S rRNA gene count = number of *Dhc* cells

The 16S rRNA molecule has insufficient information to infer physiological traits

Dhc Reductive Dehalogenases



tceA



Dehalococcoides ethenogenes strain 195

Dehalococcoides sp. strain FL2

Dehalococcoides sp. strain BAV1

Dehalococcoides sp. strain VS

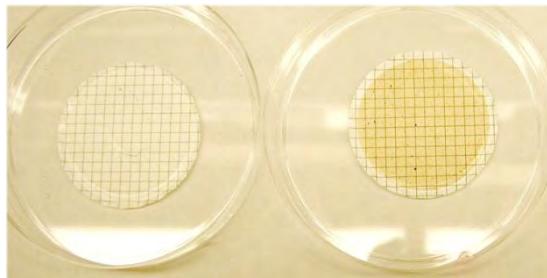
Dehalococcoides sp. strain GT

Dehalococcoides sp. strain KB-1/VS

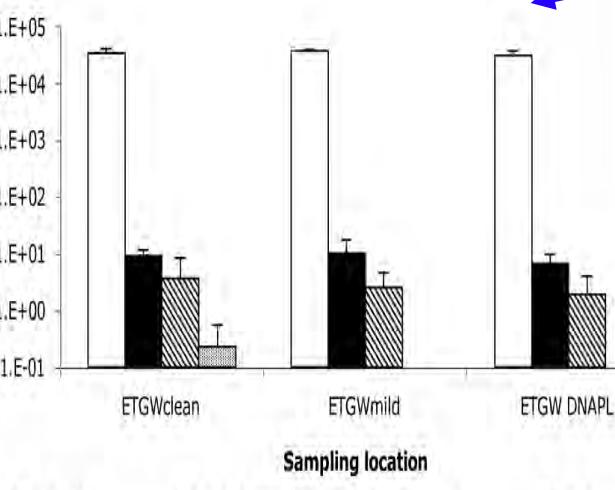
bvcA

vcrA

qPCR Sensitivity: Detection vs. Quantification



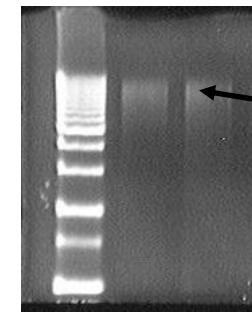
Extract Community DNA



qPCR

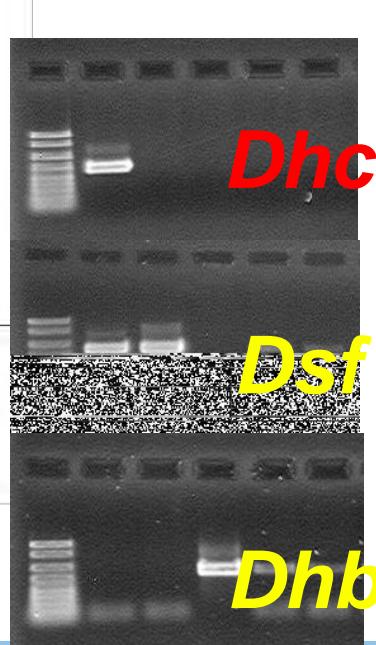


PCR

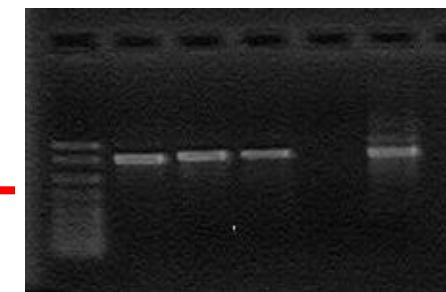


Genomic
DNA

Amplification with universal
16S rRNA gene-targeted
primers (for nested PCR)



Dechlorinator
targeted primers



Sensitive detection
of dechlorinating bacteria
(~10¹ copies/L)

Sensitive quantification
of dechlorinating bacteria
(~10³ copies/L)

Dhc 16S rRNA gene copies per L	Interpretation
10^3 or lower	Suboptimal Dhc to sustain dechlorination rates
$10^4 - 10^6$	May sustain appreciable dechlorination rates
10^7 or greater	Often associated with high rates of dechlorination and ethene production

MBT Sampling

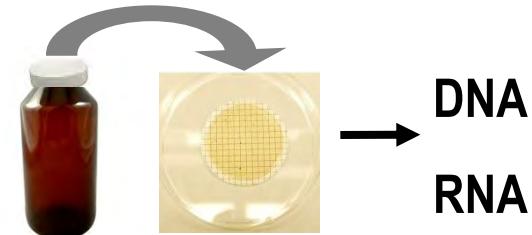
- **Groundwater sampling preferred**
 - Difficulties with repetitive soil sampling
 - Spatial variability in soil
- **Sampling method influences results**
- **Use SOP that complements VOC sampling methods**



Groundwater Sampling

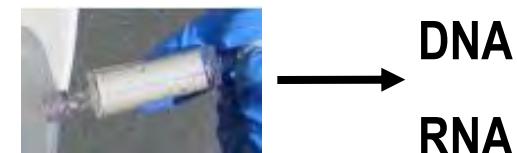
Shipping groundwater samples is problematic

- Heavy, costly
- Leakage/breakage
- Biomarker integrity
- Groundwater disposal



Improved procedure: Filtration in the field

- Economical
- No leakage/breakage
- Biomarker stability?
- GW remains on-site



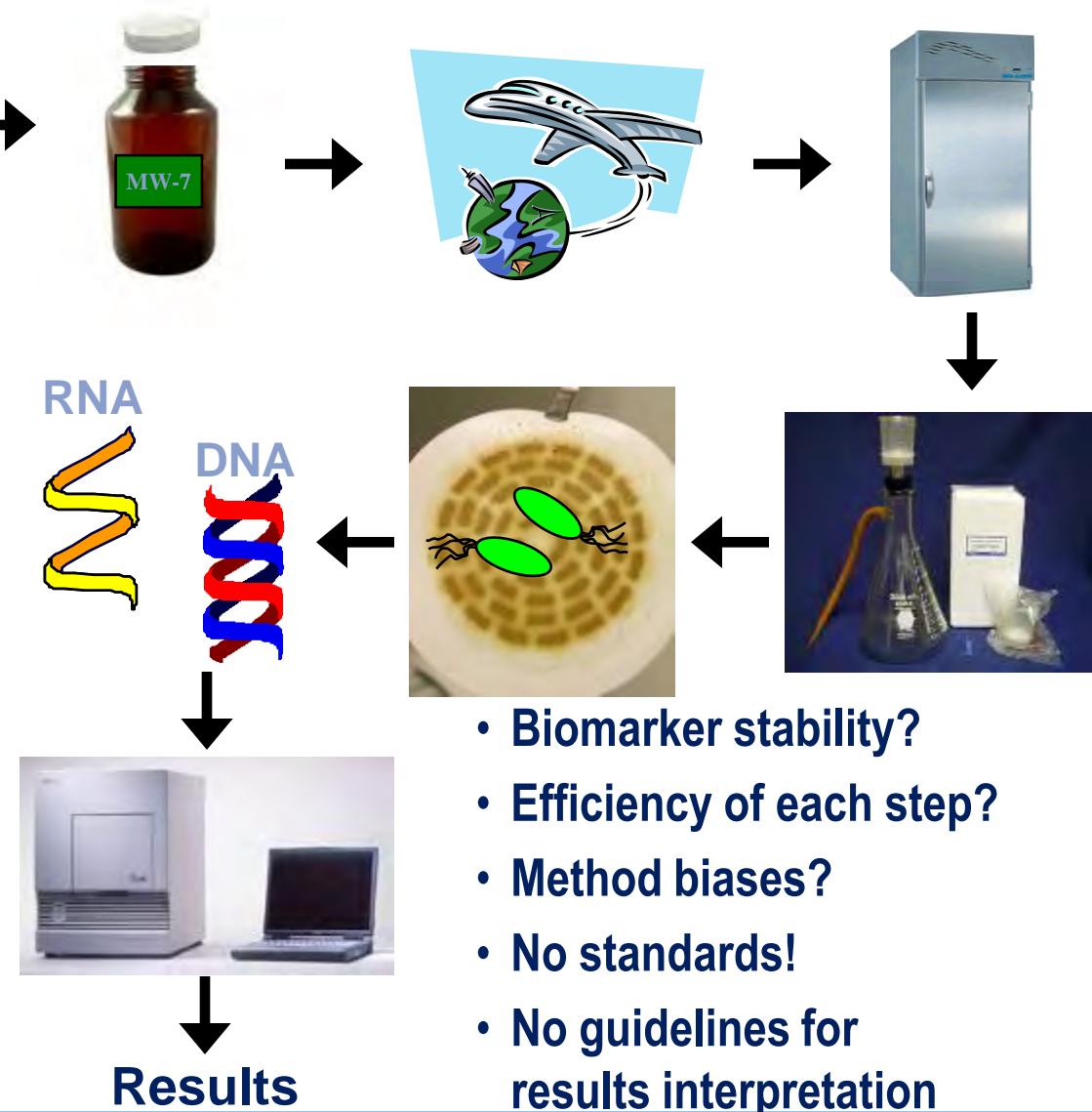
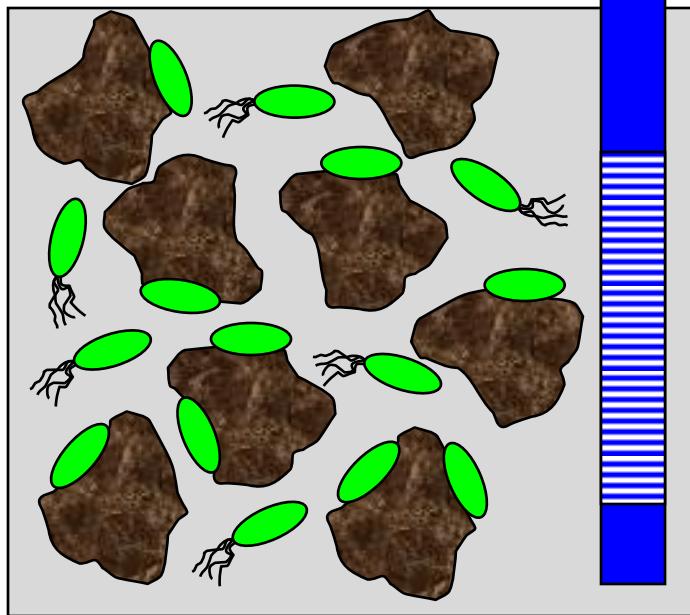
It is not practical to perform vacuum filtration in the field



ESTCP Project ER-0518:
Sterivex™ filters
are a viable alternative!

Sampling Considerations

- Sampling biases
- Ratio of planktonic vs. attached cells

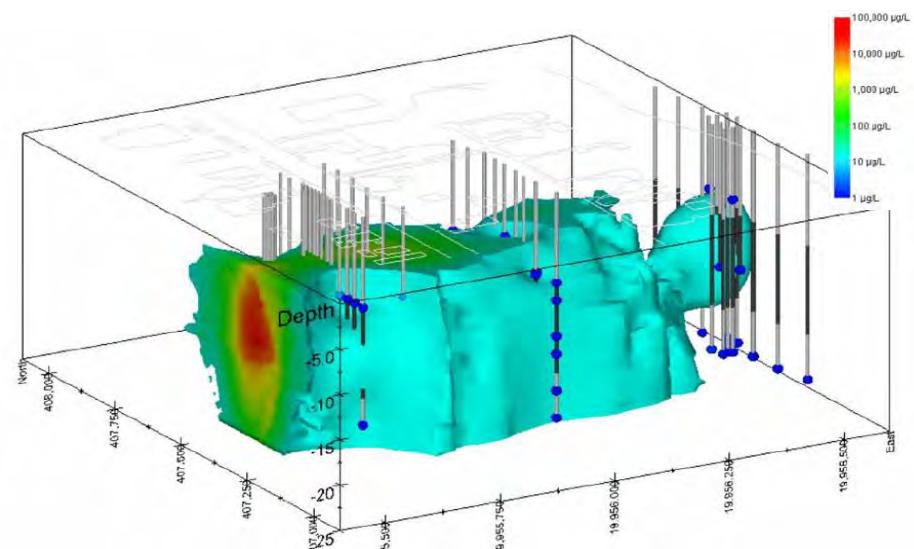


- **Low-flow purge**
 - Wait for stabilization of geochemical parameters to obtain a sample representative of formation groundwater
- **Surging**
 - Increases particulate matter in sample for recovery of associated (i.e., attached) biomass
- **Field filtration**
 - Sterivex™ filters for biomass collection in the field
 - Economical, no leakage/breakage, groundwater remains onsite
- **Shipping**
 - Secure samples for overnight shipment to laboratory
 - Maintain samples at 4°C

Sampling protocol should be defined and maintained for the duration of the monitoring effort for a particular site

Sampling Locations

- Key sampling locations should include
 - Source area(s)
 - Downgradient plume locations where biodeg products observed
 - Vertical stratification
 - Where possible, use discrete sampling zones and avoid sampling wells with extended screen intervals



- **Seasonal variability**
 - Geochemical conditions and biomarker abundance can be affected by seasonal changes (e.g., rain events, temperature changes, etc.)...be aware!
- **Bioremediation field implementation**
 - Baseline and 1-2 months after injections
 - Quarterly in first 12-18 months
 - Collect with VOC, geochemical and TOC data

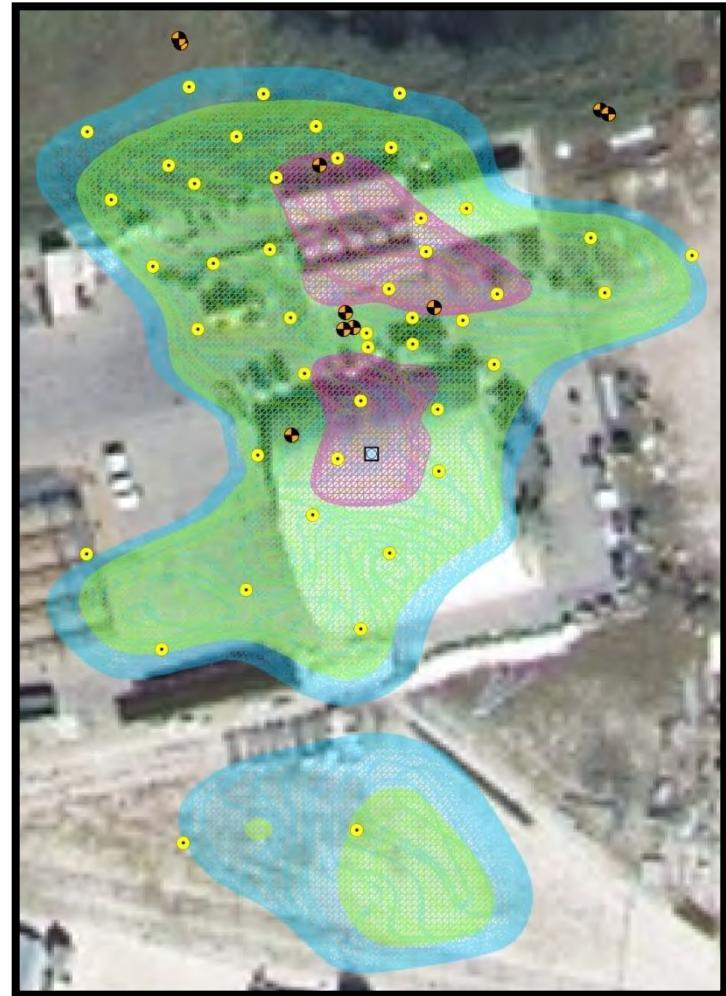
- **Field labor**
 - Biomass collection can be performed concurrently with sampling events planned for assessment of contaminants
 - Minimal additional time is needed for collection of samples for biomarker analysis
- **Laboratory**
 - Microbiology labs specializing in biomarker analysis are typically independent from chemical laboratories used for other analyses
 - Typical cost for quantification of *Dehalococcoides* in a sample of groundwater is approximately \$250

- **Unnecessary bench tests**
- **Poorly designed pilot tests**
- **Inefficient full-scale treatment**
 - Application of bioaugmentation and/or biostimulation when MNA would be appropriate
 - Bioaugmentation when sufficient Dhc are present to meet remediation goals
 - Failure to bioaugment when Dhc populations are insufficient

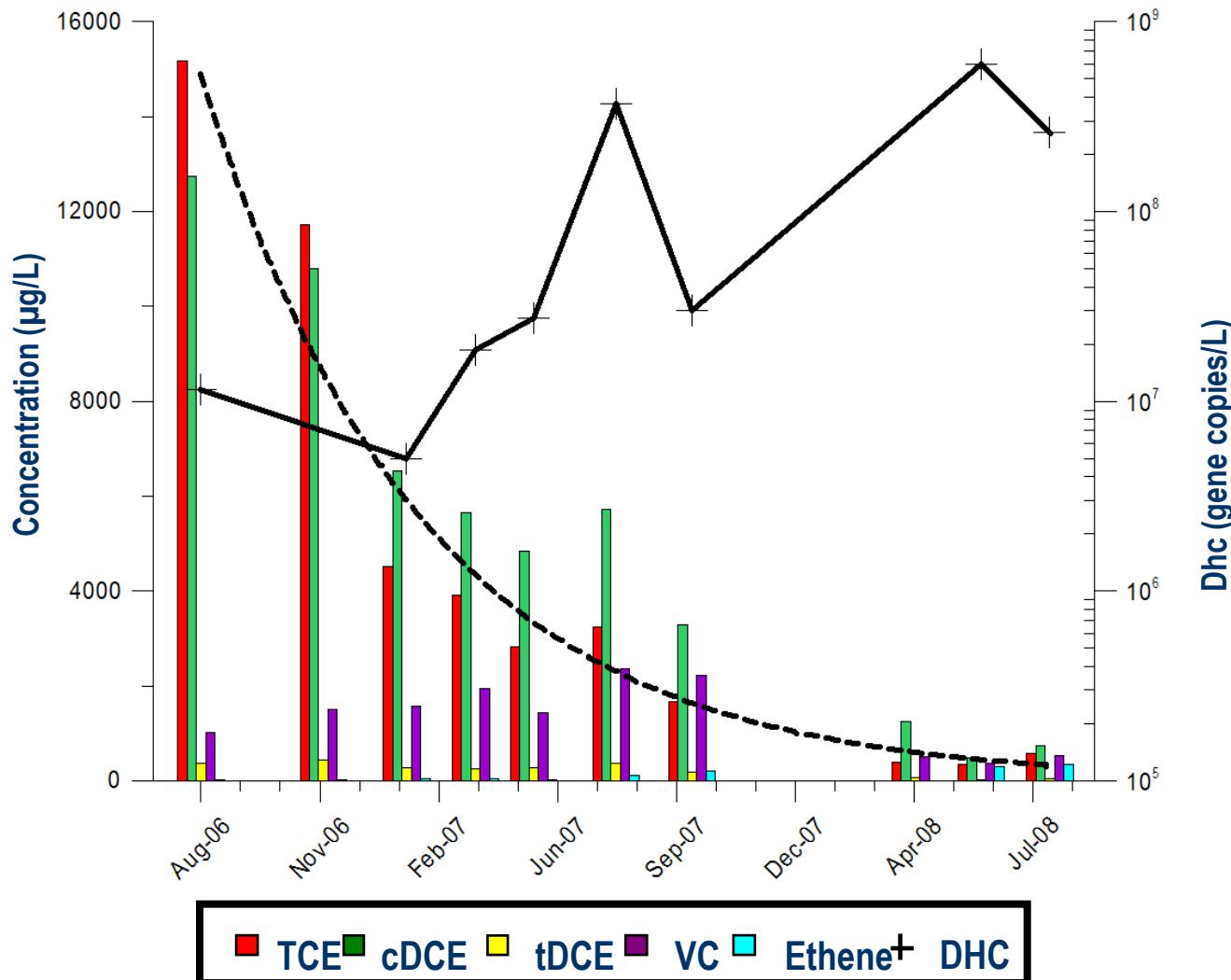
- **TCE Source Area**
 - (~4,000 gal release 1960's)
- **Biostimulation**
 - Ethyl lactate
- **Performance monitoring**
 - Every other month
 - TCE, cDCE, VC, ethene
 - Dhc and vcrA
 - ~6 data points from each well

● Monitoring Well
● Injection Well

■ 30,000 µg/L TCE
■ 3,000 µg/L TCE
■ 1,000 µg/L TCE

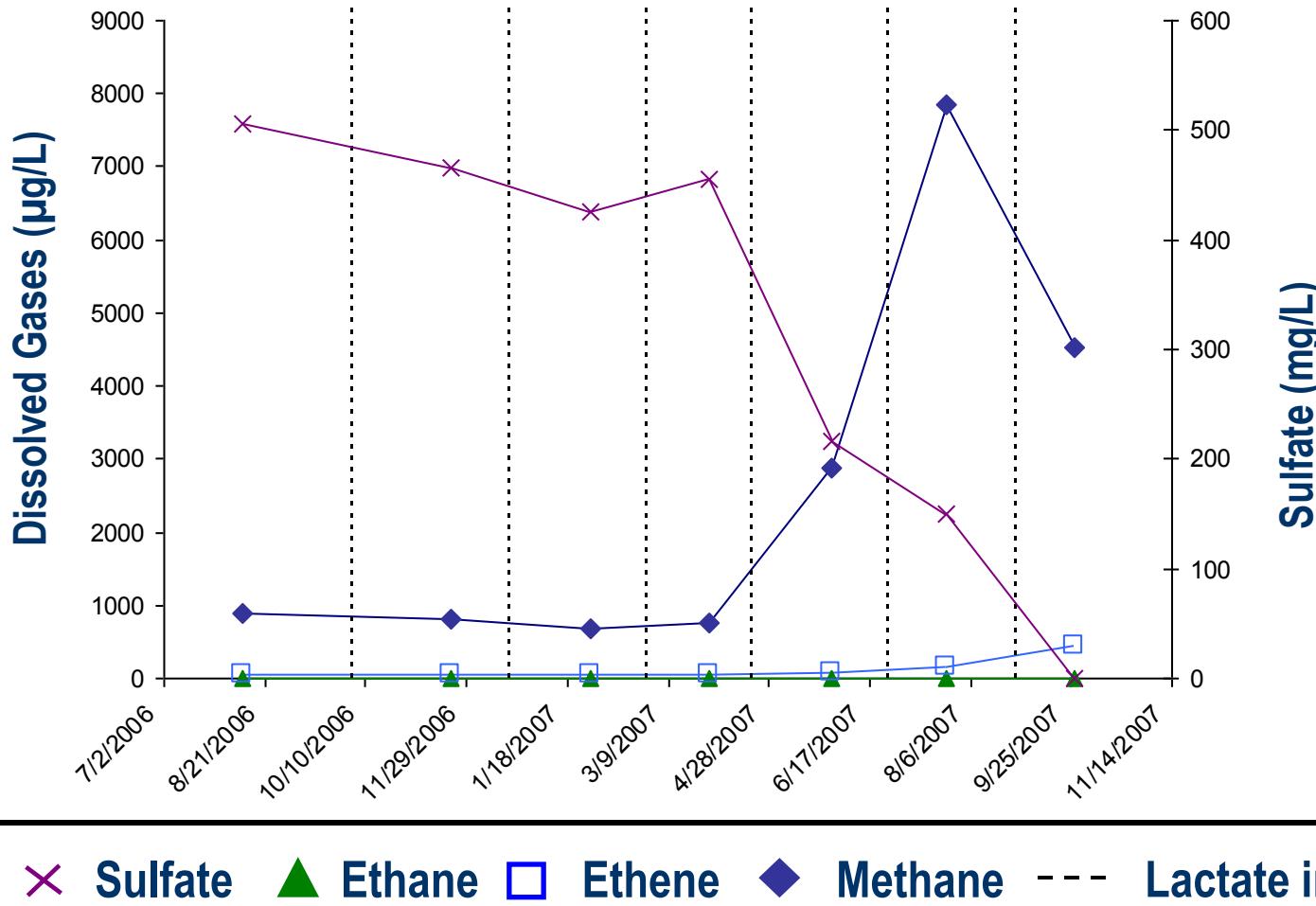


Groundwater VOCs and Dhc: SAMW-02



Dhc data
indicated no
need to
bioaugment!

Groundwater DHGs and Sulfate: SAMW-02



**Sulfate did not
inhibit
reductive
dechlorination!**

Spearman Test



• Correlation results:

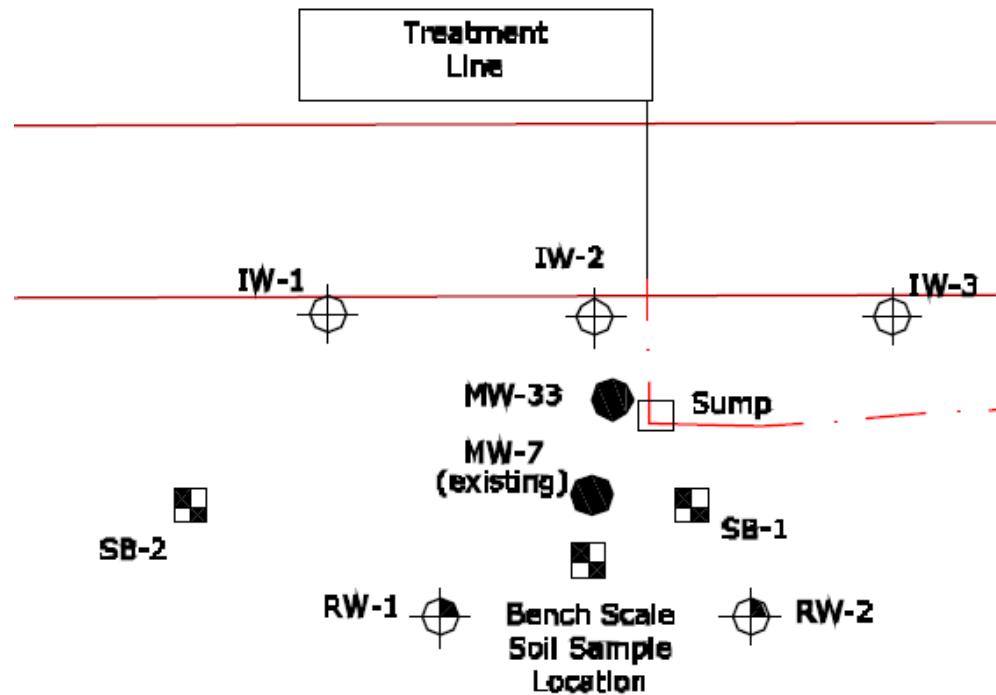
- *Dhc* or *vcrA* to TCE, DCE or VC
 - Weak correlation ($r_s < 0.33$) for all comparisons
- *Dhc* or *vcrA* to ethene
 - *Dhc* to ethene = strong correlation ($r_s = 0.66$; $n = 10$; $p = 0.05$)
 - *vcrA* to ethene = strong correlation ($r_s = 0.67$; $n = 10$; $p = 0.05$)

Spearman Test

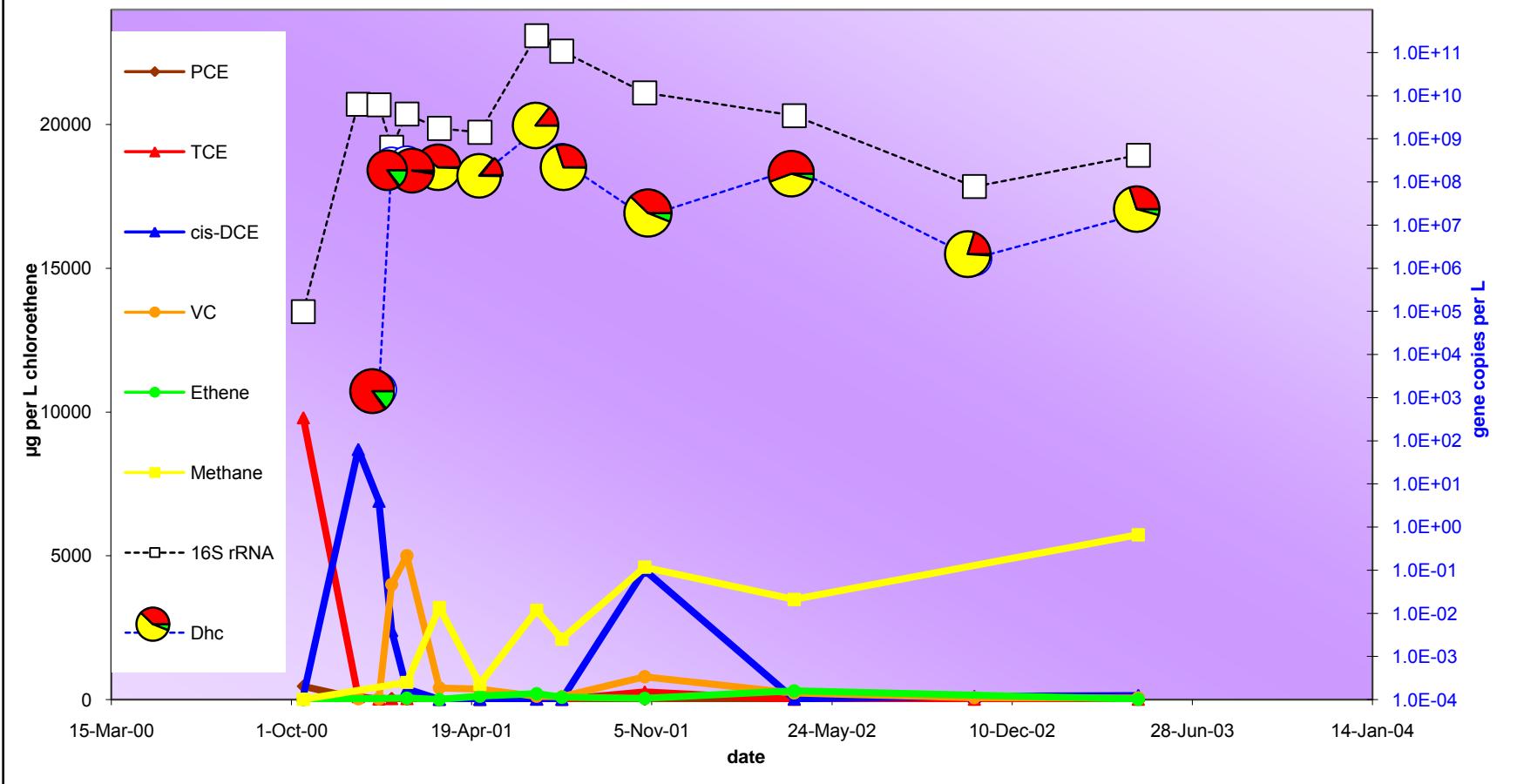


- Correlation results:
 - Strong correlations ($r_s = 1.00$) for all comparisons to *Dhc*
 - Medium correlations ($r_s = 0.50$) for all comparisons to *vcrA*
- Limited validity to results (only three data points)

- TCE Source (18,000 ppb)
- Bioaugmentation Pilot Testing
 - 3 injection wells and 2 recovery wells oriented perpendicular to the prevailing direction of groundwater flow (southwest)
 - Soluble electron donor (lactate) and dechlorinating culture distributed by recirc

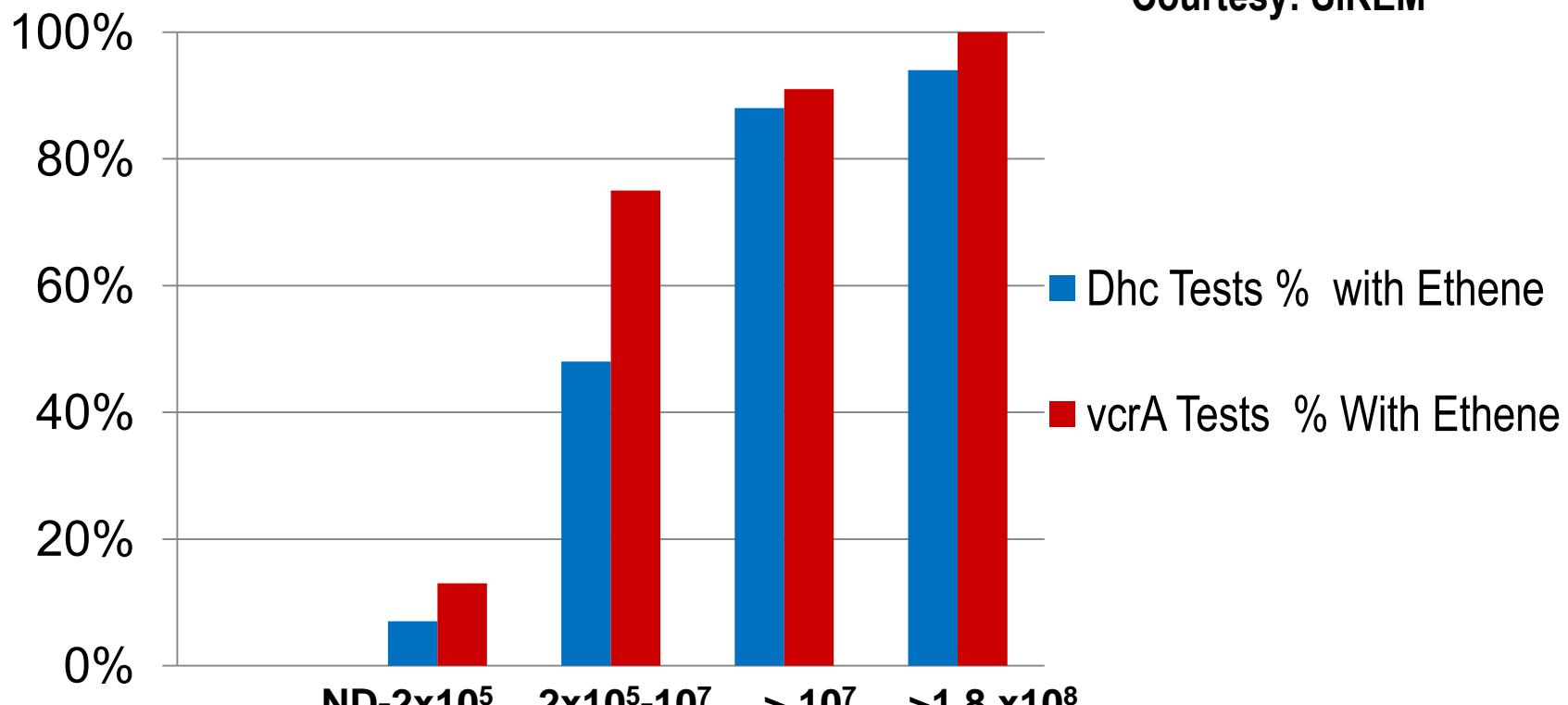


Dehalococcoides and chloroethenes



- *Dhc* or *RDases* to VOCs
 - No correlations to *Dhc*
 - Strong correlation of *bvcA* ($rs = 0$, $n = 10$, $p = 0.02$)
 - Strong correlation of *vcrA* to cDCE ($rs = -0.80$, $n = 10$, $p = 0.01$)
 - Strong correlation of *tceA* to VC ($rs = 0.76$, $n = 10$, $p = 0.02$)
 - No other correlations identified

Courtesy: SiREM



For *vcrA* testing:

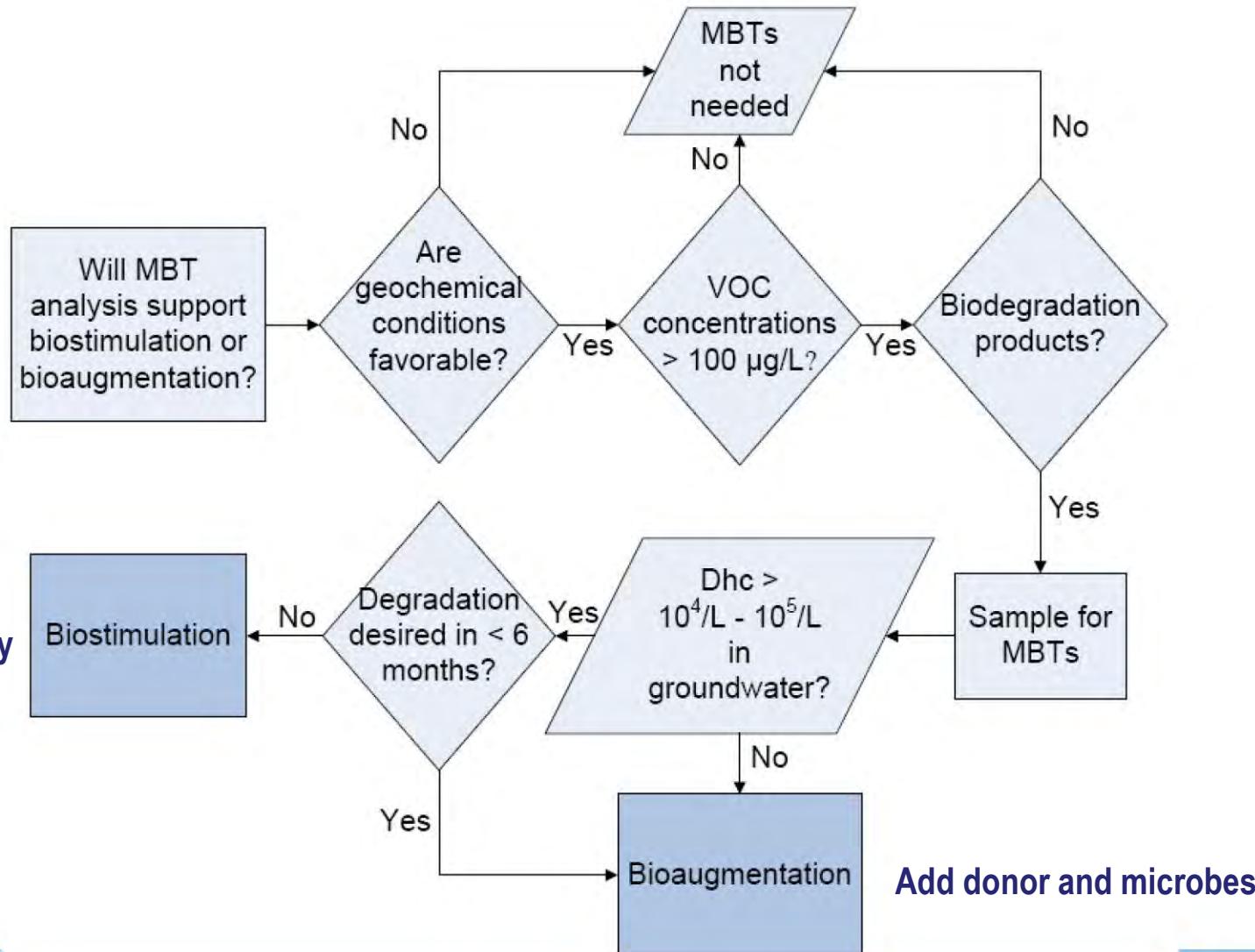
- below 2×10^5 /L ethene not normally detected
- above 1×10^7 /L ethene commonly detected
- above 1.8×10^8 /L ethene always detected

Gene copies/L

N= 121 Samples *vcrA*

N=244 samples Dhc

Biostimulation/Bioaugmentation Flowchart



Dhc 16S rRNA gene copies per L	Interpretation
10^3 or lower	Suboptimal Dhc to sustain dechlorination rates
$10^4 - 10^6$	May sustain appreciable dechlorination rates
10^7 or greater	Often associated with high rates of dechlorination and ethene production

- MBTs are valuable tools to monitor biodegradation of chlorinated ethenes
- SOPs are available for MBT sampling. Field filtration is reliable.
- Biomarker genes (*bvcA*, *vcrA*) are indicators of field dechlorination activity
- Rules of thumb and draft guidance are available
- Understand limitations of the data

Acknowledgments

Geosyntec

Dr. Rebecca Daprato

Georgia Tech

Dr. Wayne Amber

Tetra Tech
NAVFAC

Dr. Frank Löffler

Dr. Kirsti Ritalahti

Keith Henn

Carmen Lebrón

**Support provided by ESTCP project ER-0518 and
NAVFAC SE**